Histologic Study of Wound Contraction in the Rabbit *

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Wound contraction, a phenomenon occurring during the healing of full-thickness skin defects, consists of the inward movement of the intact skin margins resulting in a reduction in the wound area. As less regenerated tissue is needed for repair, the defect will be reconstituted with lesser anatomical derangement and better functional restoration. This process is more evident in animals [26] but plays a role in certain parts of the body in man.[4, 13]

The mechanism responsible for contraction is not definitely established. The many experimental investigations have studied mainly the macroscopic features of the healing of different types of excisional wounds. Microscopic observations in relation to contraction have been recorded,[5, 7, 11, 12, 19, 21, 22, 26] but we are not aware of a detailed study. The present investigation therefore endeavored to correlate histologic and macroscopic features of contracting wounds.

Material and Methods

Adult male rabbits were kept under standard conditions and fed Purina pellets, raw vegetables and water. Intravenous nembutal, supplemented by open-drip ether, was used for anesthesia. The technics of operation, photography and measurements have been previously described.[24] Identical 5 × 5 cm. full-thickness excisional skin wounds with 2 × 2 cm. islands of intact skin at their center were produced in the flank of 18 animals; the panniculus carnosus was left intact. The wounds were not dressed. In one animal the wound was excised and examined, as described below, immediately after it was produced. A wound was similarly excised in one of the other animals on alternate days up to Day 30, one was excised on Day 40 and one at Day 100. The excision consisted of the entire wound, with a standard area of surrounding skin and the underlying panniculus carnosus. The excised specimens were mounted on cardboard, so that they occupied exactly the same area as they had *in vivo,* and fixed in formalin. Seven standard blocks of tissue were taken for histologic examination from each of the 18 specimens. The blocks were embedded so that the microscopic sections were of a vertical plane in four and a horizontal in three. They were stained with hematoxylin and eosin, and a polarizing microscope was employed to identify and determine the direction of the collagen fibers.

Results

We had previously found [24] that healing of this type of excisional wound in the rabbit was divisible into five macroscopic phases (Fig. 1), and the results will be presented in relation to these.

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Phase I: Immediate Retraction: Day 0. Following excision, all the intact skin margins promptly retract away from the wound cavity, resulting in an increase in the width of the wound cavity (Fig. 1, 2a). Histologic examination shows the cut edges of both the wound and island to slope towards the wound cavity, indicating that the superficial is affected to a greater degree than the deeper part of the wound. The floor of the wound cavity consists of a thin layer of connective tissue covering the panniculus carnosus.

Phase II: Early Reduction: Day 1–2. The wound cavity becomes covered by a scab and the wound area is reduced (Fig. 1). Histologic examination (Fig. 3) shows the island partly undermind by the wound cavity. The scab consists of a superficial zone of fibrin and cellular debris overlying a layer of connective tissue that has undergone ischemic necrosis; intact collagen fibers can be traced from this necrotic layer into the deeper viable connective tissue.

Phase III: Latent Period: Days 3–7. The macroscopic features of the wound change very little (Fig. 1); toward the end of the phase, the scab becomes fragmented. Histologic examination at

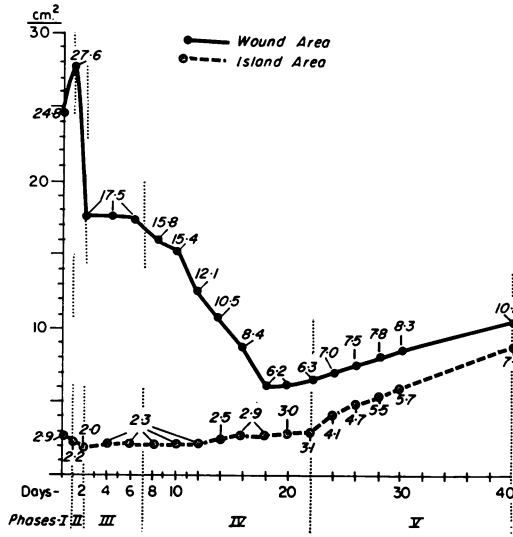

FIG. 1. Representation of macroscopic phases of healing of wound illustrated in Figure 2. Phase I: Immediate retraction. Phase II: Early reduction. Phase III: *Latent* period. Phase IV: Contraction. Phase V: Functional adaptation. Recorded wound area includes island area. Difference between wound and island areas represents area of wound cavity.

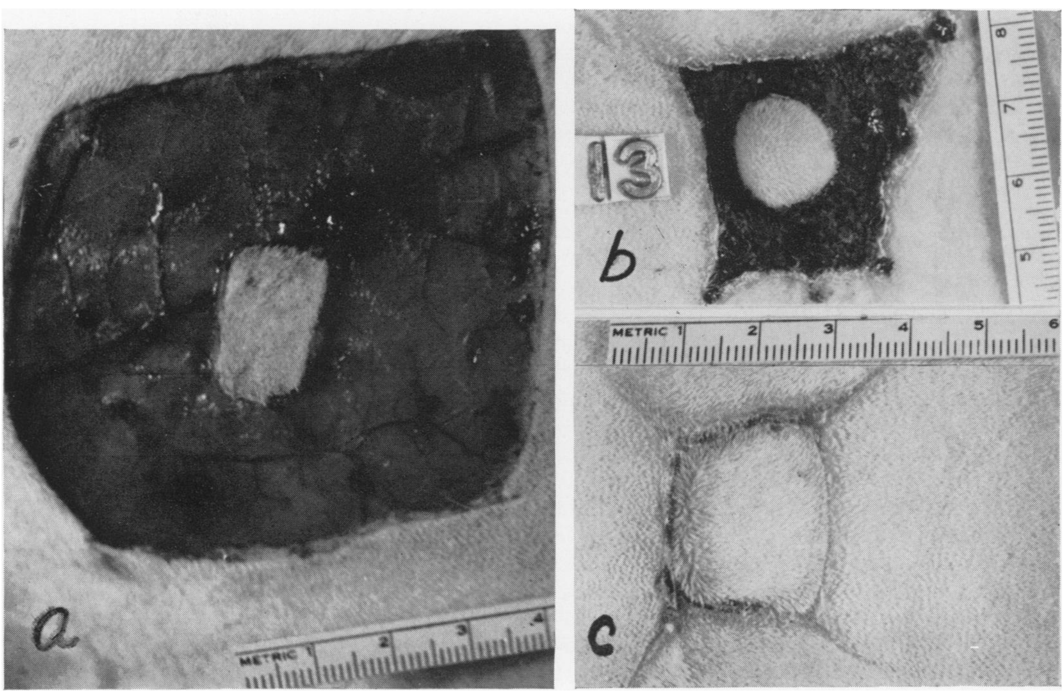

FIG. 2. Healing of a 5 × 5 cm. excisional wound with a 2 × 2 cm. island of intact skin at its center: a) Day 0: Immediate retraction after wounding has resulted in enlargement of the wound and decrease in size of the island. b) Day 13: During contraction the wound margins move inward, approaching the periphery of the island, reducing the wound area. The island, which is also reduced, is rounded and bulges above the surface. c) Day 20: The wound cavity is almost completely closed and the island has flattened and enlarged. The wound has assumed a stellate shape.

FIG. 3. Day 2 (end of phase of early reduction). Island is undermined by wound cavity, which is covered by a scab consisting of two layers described in text (from × 40).

Day 6 (Fig. 5) shows the width of the wound cavity unchanged from Day 2. Migrating epithelium covers the cut edges and the adjacent periphery of the wound bed, undermining the scab. Deep to the latter there is a small amount of young granulation tissue. In this the orientation of the fibroblasts is multidirectional, with the exception that at the base of the wound margins they were found to be mainly at right angles to the periphery of the wound.

Phase IV: Contraction: Days 8–20. Macroscopically, the wound margins move contripetally, causing a decrease in the wound area (Fig. 1, 2b); the island margins also move centripetally, but to a much lesser degree. (When related to the wound cavity, these two movements differ: the wound margins move toward the cavity while the island margins move away from it.) The wound margins reach almost to the periphery of the island by Day 18 and almost close the wound cavity. The wound assumes a stellate shape. After Day 16 there is a reversal of the direction of movement and both the wound and island margins move centrifugally. This continues during the remainder of the phase so that both the wound and island gradually increase in area. Contraction is considered terminated when no further significant decrease of the area of the wound cavity occurs. At this time the wound cavity is macroscopically closed (Fig. 2c). Histologic examination at Day 8 shows the scab to be fragmented. Almost no inflammatory reaction remains in the wound margins or the islands; an acute inflammatory cell reaction is present at the junction of the scab and the underlying granulation tissue. The latter has increased in amount and thin collagen fibers have made their appearance. Distinct orientation of the fibroblasts at the base of the wound margin is no longer detectable. At Day 10 (Fig. 4), the wound cavity is reduced in width, partly covered by epithelium and filled with regenerated connective tissue in which maturation is occurring. The island margins are undermined by the regenerate tissue. In vertical sections, the newly formed collagen fibers have a multidirectional orientation. In sections in the horizontal plane of the skin, a predominant circumferential orientation parallel to the periphery of the island is found; some other fibers are directed in a radial fashion from the wound bed to the wound corners. At Day 14 (Fig. 6a) the bulging of the island has increased and its base reduced in width by the undermining wound regenerate. There has been a further reduction in the width of the wound cavity between the two intact coriums; the regenerated tissue has the outline of a sword-fish (Fig. 6b) with the superficial portion only 0.3 cm. in width, while the deepest part is approximately as wide as this side of the wound cavity when originally produced. Collagen fibers in the regenerated tissue blend with the fibers covering the panniculus carnosus, but cannot be demonstrated to pass into the intact corium of the wound margin or island. The hair follicles in the wound margin are spread apart. At Day 18 (Fig. 7), the island has flattened and the coriums of the wound margin and island are less than 0.2 cm. apart. In horizontal sections the fibers and cells of the regenerate show a persistence of the circumferential and radial orientation found at Day 10 (Fig. 8).

Phase V: Functional adaptation: Day 22 and beyond. A gradual increase in the size of the island occurs so that by Day 40 it is twice as large as originally produced (Fig. 1). Concomitantly, the perimeter of the island and the arms of the scar became elongated and the wound margins expand back toward their original position. Beyond Day 40 no additional significant macroscopic changes can be detected in the wound. Histologic examination at Day 30 (Fig. 9) shows further maturation of the regenerate and epithelium. The coapted wound and island margins have shifted centrifugally over the regenerate, and to a lesser extent the regenerate itself has also shifted centrifugally. The hair follicles in both the island and in the wound margins are now spread apart. On Day 40 the wound shows little change; the regenerate is still distinct from the pre-existing tissue. At Day 100 (Fig. 10) the site of the previous wound cavity at the level of the corium can no longer be identified. However, a distinct scar as wide as the originally produced wound cavity persists deep to this remodelled corium and collagen fibers are now found connecting the two.

Discussion

The findings are outlined in schematic form in Figure 11. It can be seen that, in addition to regeneration, a complex series

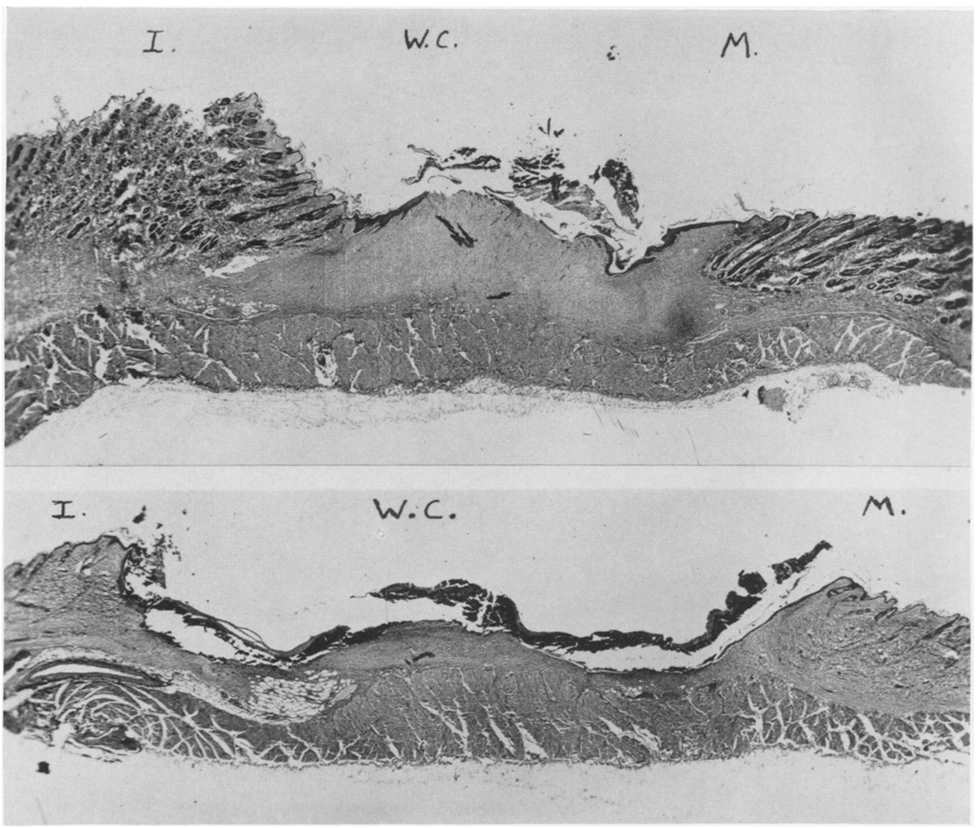

FIG. 4. (Top) Day 10. The wound cavity is reduced in width and partially re-epithelized. The amount of regenerate is markedly increased. The >- shaped formation, outlined in the wound margin, is formed by a bundle of fibers which becomes prominent in the sloping cut edge of the wound margins, intersecting the collagen fibers in the deep layer of the intact corium (from × 8).

FIG. 5. (Bottom) Day 6 (end of *latent* period). Sloping cut edge of the wound margin (M) is covered by migrating epithelium which undermines the scab; the latter has become fragmented. There is a small amount of young granulation tissue in the wound cavity (W.C.). The deepest part of the section consists of the intact panniculus carnosus. The island (I) is at the left (from × 8).

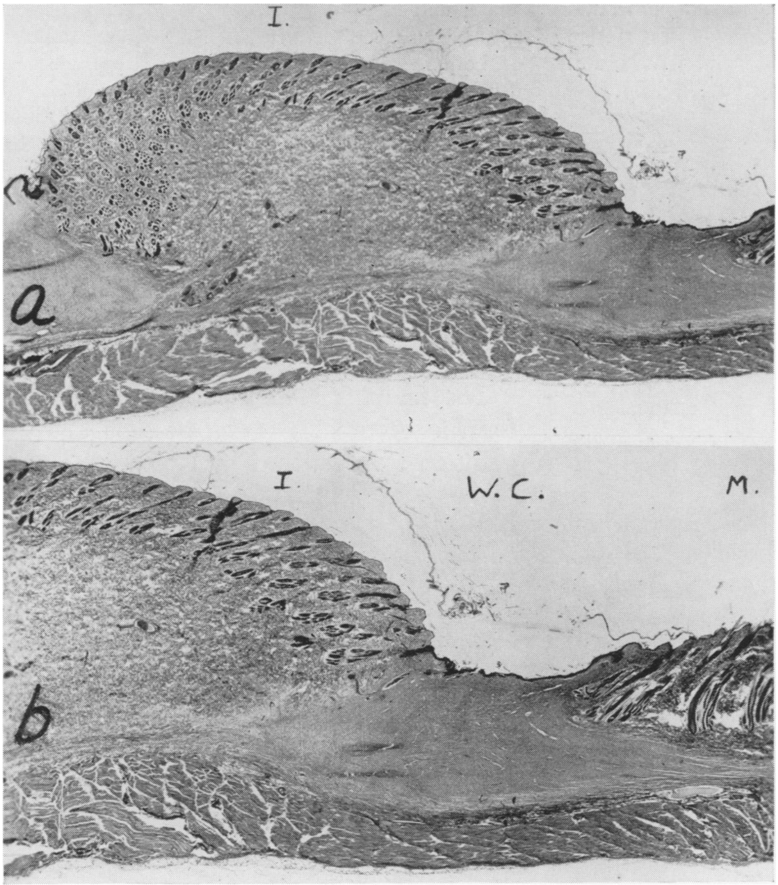

FIG. 6. Day 14. a. Island bulges above level of surrounding skin. Base has been undermined by regenerate on each side and reduced to a diameter of 0.6 cm. (originally 2 cm.) (from × 12). b. Slightly higher magnification of Figure 6a showing outline of regenerate and its independence from intact coria (from × 15).

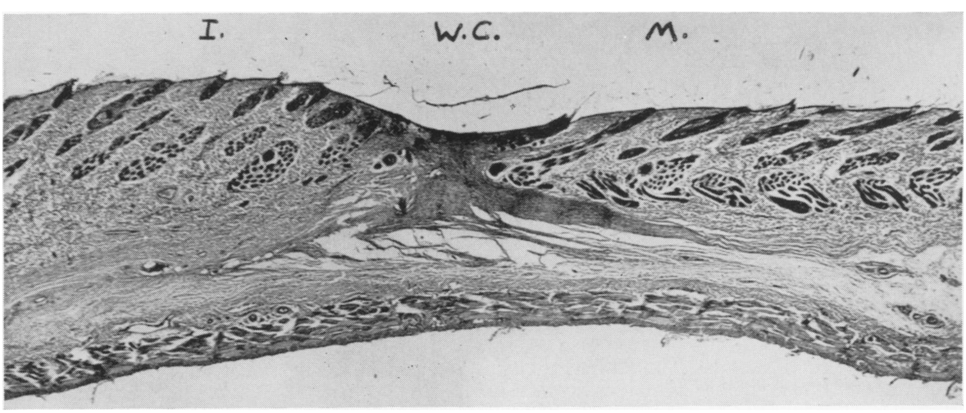

FIG. 7. Day 18. The island is now almost flat. The intact coria of the wound and island margins are separated by less than 0.2 cm. of regenerate. Comparison with Figure 4 and 6b demonstrates the extent to which the wound margin has moved over the regenerate during contraction. The twist of the hair follicles in the wound margins results from this inward movement of the corium (from × 15).

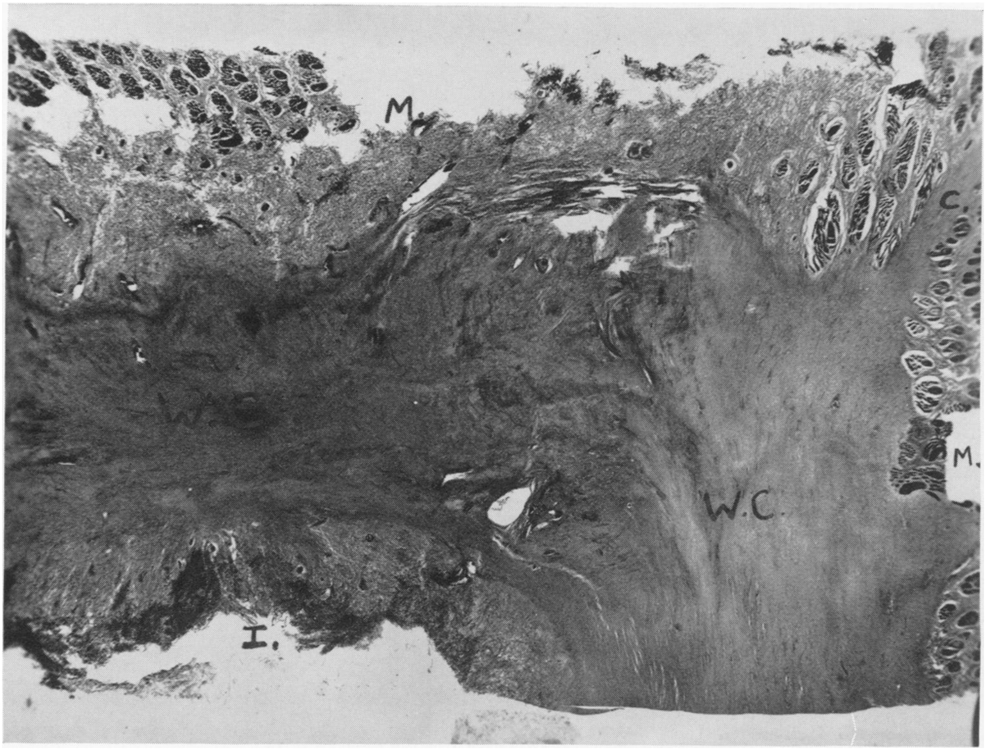

FIG. 8. Day 18. Horizontal section of the wound illustrated in Figure 7. Between the margins of the island (I) and the wound (M) is the wound cavity (W.C.) filled with regenerated connective tissue. In this regenerate, a predominant orientation of fibers and cells is circumferential around the island; radially orientated fibers pass from the main body of the regenerate to the corner of the wound (C) which represents an arm of the stellate scar (from × 8).

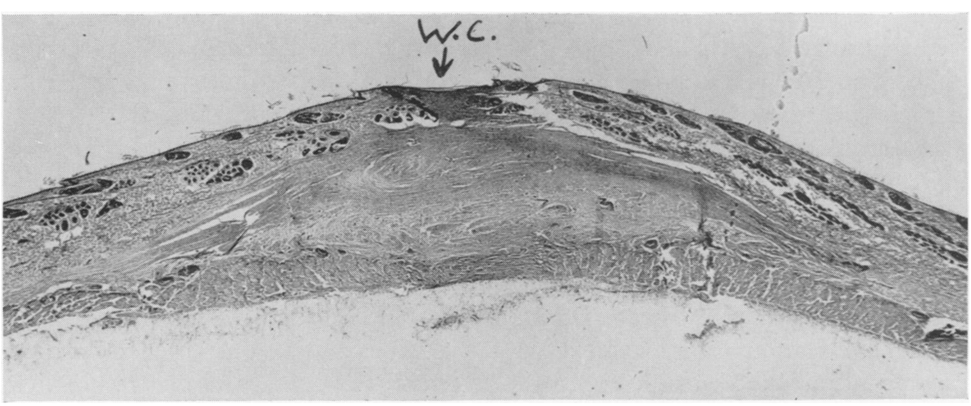

FIG. 9. Day 30. Regenerate is still quite distinct. Only a small amount remains between the two intact coria, but in the deeper part of the wound it is approximately equal in width to that of the originally produced wound cavity. The island is flat. Superficial part of the wound cavity (W.C.) now lies over the mid-point of the regenerate, indicating that the coapted coria have shifted centrifugally (from × 8).

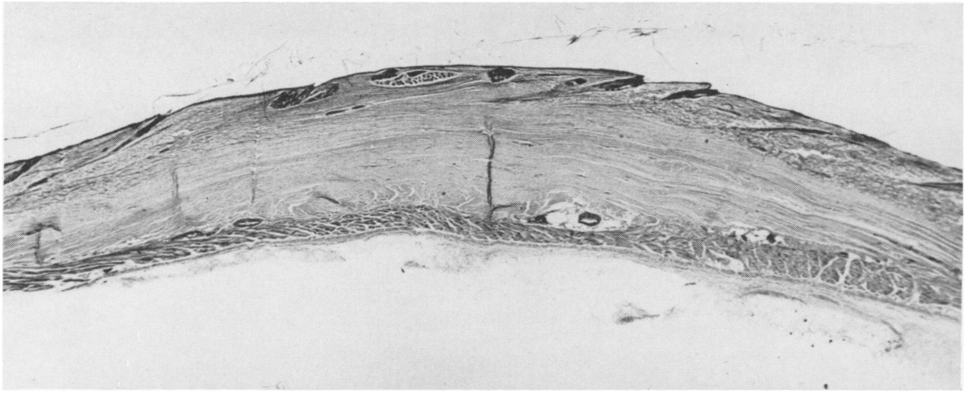

FIG. 10. Day 100. It is almost impossible to locate the site of the scar superficially. Deep to this a wide scar is still evident. Hair follicles are widely spaced (from × 8).

of movements occurs. As epithelization and connective tissue regeneration are well documented in many sources,[14,15] they have not been described in detail. Wounds with an island were chosen for study, since they have been found to magnify the changes occurring in the center of the wound and to focus the movements during healing.[5,24]

The suggested mechanisms responsible for the various phases have been recently reviewed.[24] Immediate retraction has been shown to vary with the fixation and elasticity of the skin[21] and seems best explained as a pull exerted on the wound margins by the intrinsic tension forces that Cox[10] has shown to be normally present in skin. Early reduction has been suggested by Cuthbertson[11] to result from shrinkage of the scab secondary to its dehydration. How this shrinkage is transmitted to the wound is not clear; possibly it is through the uninterrupted collagen fibers that were found to extend into the underlying structures from the necrotic connective tissue forming the deep part of the scab. During the *latent period* no gross movement occurs; however, marked histologic regeneration is found and active biochemical processes have been demonstrated.[13]

Contraction accounted for the major portion of the wound closure in this study. A number of hypotheses have been proposed regarding the mechanism: 1) raised tissue pressure in the skin margins bordering the wound, secondary either to inflammation[14] or to re-arrangement of the tissue components[26] (morphyllaxis), push the wound margins inward toward the wound cavity (*push* theory); 2) regenerating fibroblasts at the base of the intact skin margins, by retaining an embryonal-type migratory capacity, move inward and carry the margins with them[17] (*picture frame* theory); 3) changes in circumferentially oriented collagen fibers in the wound bed result in a sphincter action and close the wound[12] (*sphincter* theory); and 4) the force for contraction lies in the regenerated tissue in the wound bed and pulls the wound margins inward[3,8,9,11,20] (*pull* theory).

Experimental evidence against the first three hypotheses has been presented[3,9,11,20,24] and currently the data seem mostly to favor the *pull* hypothesis.[13] This theory was first proposed by Carrell in 1910,[8] who suggested that the force was generated by *granluous contraction* in the wound bed. For many years this was thought to result from shrinkage of the newly formed collagen fibers. However recent evidence shows this to be highly improbable.[1, 18] Alternative explanations propose that the *pull* is mediated through an action of the regenerating fibroblasts in the wound bed,[3, 9] or that it is related to both collagen formation and absorption in the wound cavity.[13, 20]

Some of the findings in the present study appear to be relevant to this problem. The effect of the contracting force on the different components of the wound varied: the entire wound with its margins and contents shifted centripetally, but to a lesser degree than the more mobile corium of the wound margin; the latter slid centripetally over the top of the regenerated tissue in the wound cavity; the base of the island decreased in size and its margins moved away from the cavity. Thus in wounds with

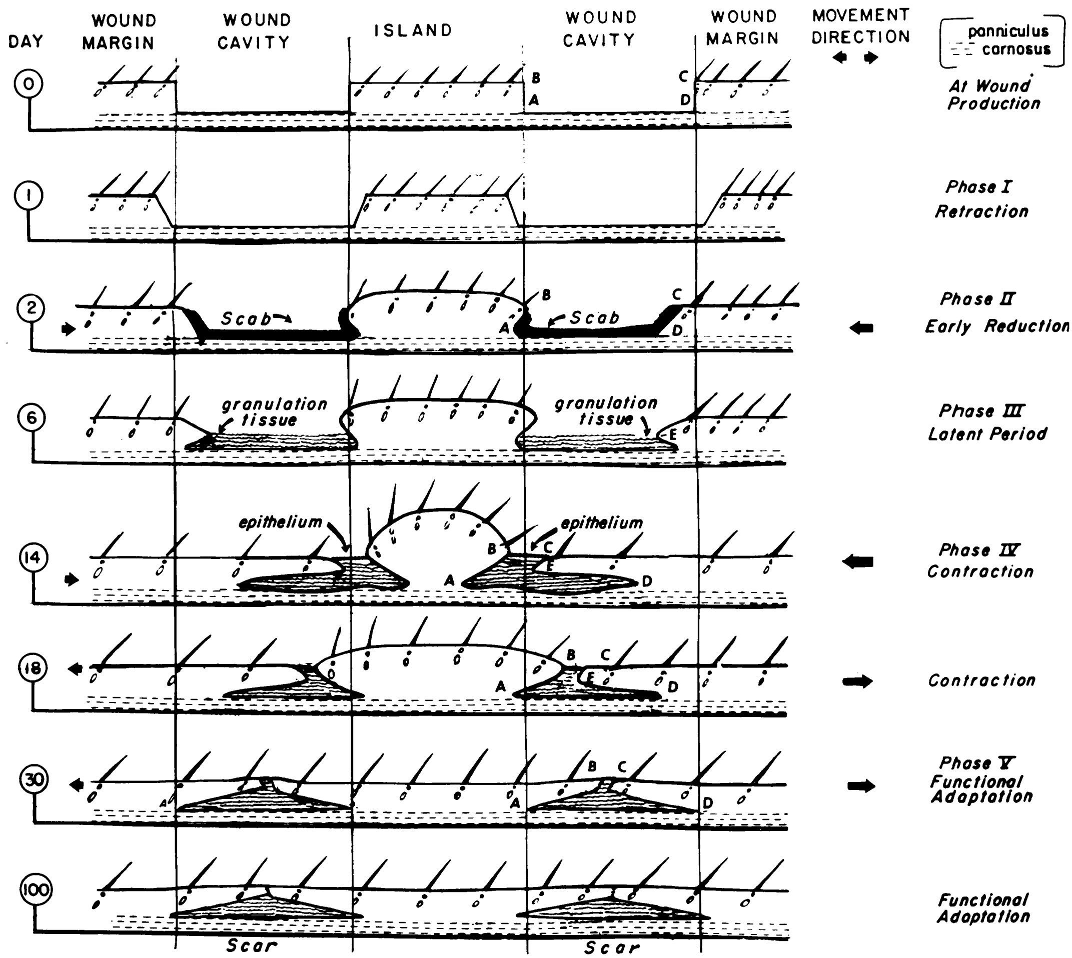

FIG. 11. Schematic representation of closure of a full-thickness excisional wound with an island. Phase I: Retraction affects mainly the superficial portion. Phase II: Wound cavity with wound margins move centripetally, undermining the island. Phase III: No gross movements occur; histologically, marked regeneration occurs. Phase IV: Composite centripetal movement occurs, consisting of a sliding of wound margins over the regenerate and a shift of wound cavity and its contents, further undermining the island. This causes the island to bulge above the surface of the surrounding skin and move away from cavity. Day 18. During the latter part there is a rapid expansion of island. Phase V: Day 30. Composite centrifugal movement occurs, consisting of a sliding of the approximated coria over the regenerate and a shift of the base of the wound cavity back to its original position. Day 100. Superficial location of the original wound is difficult to detect. There is an appreciable amount of deep scar, equal in width to the originally produced wound cavity. Hair follicles are spread apart.

islands, the contracting force is directed toward the geographical center of the wound (the center of the island), rather than to the center of the regenerated tissue in the wound cavity (represented here by a line equidistant from the wound margin and the periphery of the island). The island is only passively constricted by this force and move away from the wound cavity. This rules out both a *push* from the intact tissues, or a *picture-frame* mechanism, as both of these should result in a movement of the island margins toward the wound cavity. The >-shaped formation, which appeared in the intact corium of the wound margin (but not in the island margin) and persisted as the leading point during contraction, can also probably be related to the contracting force. The appearance of this prominent bundle of collagen fibers in the wound margin is interpreted by Weiss' observation [27] that when a hole in a net becomes smaller, the fibers of the mesh connected with its periphery become oriented perpendicular to the margin.

Although the bulk of evidence does not indicate that the force for contraction is generated at the base of the wound margins (*picture frame*), the integrity of this zone appears to be crucial for contraction, as mechanical disruption of this area arrests contraction.[17, 23] This may be explained by certain of the histologic findings. The lack of crossing fibers between the regenerate and pre-existing coriums shows that in excisional wounds connective tissue does not regenerate from the intact coriums, a finding similar to that of Gillman and Penn's [16] in incisional wounds. The lack of crossing fibers results in an independence of the corium. This independence, plus the persistence of the >-shaped formation during contraction, suggests that the contracting forces must be applied either at the tip of this formation or adjacent to it at the base of the corium. Thus interference with this area would impair the transmission of the force.

The *sphincter* theory proposed by Dann *et al.*[12] suggested that contraction would occur by virtue of shortening of the circularly arranged collagen fibers observed in simple excisional wounds. We have confirmed this arrangement in wounds with islands and it has also been noted in Sanderson-Clark Chambers.[25] However, we cannot agree with the mechanism proposed by Dann *et al.* [12] as the circular fibers only became apparent after the onset of contraction and also because contraction has been shown to occur in the absence of collagen.[1, 18] Nevertheless, as tensile forces have been shown to influence the orientation of cells and fibers in regenerating connective tissue,[6, 25] the circularly arranged fibers suggest a circumferential orientation of many of the forces acting on the wound cavity.

It is difficult to envision how, in wounds with islands, a *pull* from the regenerating connective tissue could result in a force not directed toward its own center, not cause predominantly radially arranged new fibers and not result in an enlargement of the island. A sphincter-like action residing in the wound cavity might explain the findings in the present experiment, but the nature of such a mechanism could not be identified from the present study.

The phase of functional adaptation is mainly a remodelling of the scar.[27] The centrifugal movements of the coapted wound and island margins, that replace the centripetal movements late in the phase of contraction, continues into this phase. Histologic examination showed that the centrifugal movement is also a composite one; mainly it is a sliding of the approximated corium over the regenerated tissue, with a lesser shift of the base of the wound cavity back to its original position. Although not definitely established, the centrifugal movements are probably effected by the intrinsic tension forces in the unwounded skin acting on the contracted wound and increased by the process of contraction.[5] Billingham and Medawar[5] have shown that a restoration of normal structure occurs in the tissue surrounding the wound by means of intussceptive growth. In agreement with others,[11, 21] we found that although the intact coriums were approximated and remodelled, an appreciable and distinct scar remained in the deeper part of the wound. Abercrombie[2] has presented biochemical data suggesting that the amount of scar actually increases.

Summary

A study was carried out in rabbits to correlate the histologic and macroscopic features of the healing of an excisional wound with an island of intact skin at its center. Contraction accounted for the major portion of wound closure and was found to be a composite centripetal movement consisting of a sliding of the corium of the wound margins over the regenerated tissue, and a shift of the entire wound cavity with its margins and contents. The newly formed collagen fibers in the regenerated tissue showed a predominant circumferential arrangement, indicating that the forces acting on the wound cavity were disposed mainly in a circular fashion. Although contraction reduced the amount of regenerated tissue in the superficial part of the wound cavity, a scar as large as the original cavity remained in the deeper part.

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